IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Reissue Applicants	:	Hiroyuki Nakane, Chikara Ohto, Shinichi Ohnuma, Kazutake Hirooka, Tokuzo Nishino
Reissue Serial No.	:	09/902,651
Reissue Application Filed	:	July 12, 2001
Patent No.	:	5,935,832
Issued	:	August 10, 1999
Title	:	FARNESYL DIPHOSPHATE SYNTHASE
Examiner	:	STEADMAN, David J.
Art Unit	:	1656

Box REISSUE
Assistant Commissioner for Patents
Washington, D.C. 20231

SUBSTITUTE REISSUE APPLICATION DECLARATION BY THE INVENTORS

We, Hiroyuki Nakane, Chikara Ohto, Shinichi Ohnuma, Kazutake Hirooka and Tokuzo Nishino, hereby declare that:

1. Each inventor's residence, mailing address and citizenship are stated below next to their name.

2. We believe the inventors named below to be the original and first inventors of the subject matter which is described and claimed in patent number 5,935,832 (the '832 patent), granted August 10, 1999 and for which a reissue patent is sought on the invention entitled "Farnesyl Diphosphate Synthase," the specification of which was filed on July 12, 2001 as reissue application number 09/902,651 and was amended on July 12, 2001, March 18, 2005, November 7, 2005, March 31, 2006, June 2, 2006, June 23, 2006, December 22, 2006, June 29, 2007, October 20, 2007, June 3, 2008 and March 6, 2009.

3. We have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

4. We acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56.

5. We hereby claim the foreign priority benefit under 35 U.S.C. 119 of the Japanese application listed below.

Foreign Application Priority Information:

Serial No.	Filing Date
Japan 8-213211	Jul. 24, 1996

6. We verily believe the original patent to be wholly or partly inoperative or invalid, for the reason described below: by reason of the applicants claiming more or less than they had the right to claim in the patent.

7. At least one error upon which reissue is based is described below.

Claim 1 of the '832 patent is directed to a mutant prenyl diphosphate synthase, and the claim is reproduced below.

Claim 1. A mutant prenyl diphosphate synthase having a modified amino acid sequence, wherein

said mutant diphosphate synthase comprises an aspartic acid-rich domain having the sequence, $D_1D_2X_1X_2(X_3X_4)D_3$, in region II of said mutant prenyl diphosphate synthase,

wherein each of D_1, D_2, and D_3 denote an aspartic acid residue; X_1, X_2, X_3, and X_4 are each independently any amino acid and X_3 and X_4 are each optionally independently present in the aspartic acid rich domain, and wherein

said mutant prenyl disphosphate synthase comprises (1) at least one amino acid substitution, said at least one amino acid substitution located at least one amino acid position selected from (a) an amino acid between D_1 and the amino acid residue at the fifth position upstream of D_1 and (b) the amino acid residue located one amino acid position upstream of D_3; (2) at least one

additional amino acid inserted between D_3 and the first amino acid upstream of D_3; or a combination of (2) and (3);

wherein said mutant prenyl diphosphate synthase synthesizes prenyl diphosphate which is shorter than prenyl diphosphate synthesized by a corresponding wild-type enzyme.

Claim 9 of the '832 patent depends on claim 1, and is reproduced below.

Claim 9 A mutant prenyl diphosphate synthase according to claim 1 wherein at least one amino acid selected from phenylalanine at position 77, threonine at position 78, valine at position 80, histidine at position 81, and isoleucine at position 84 has been substituted by another amino acid, and/or two amino acids have been inserted in between isoleucine at position 84 and methionine at position 85 in the geranylgeranyl diphosphate synthase as set forth in SEQ ID NO: 1, wherein the phenyl alanine at position 77 has been replaced with tyrosine, the threonine at position 78 has been replaced with phenylalanine or serine, the valine at position 80 has been replaced with isoleucine, the histidine at position 81 has been replaced with leucine or alanine, or the isoleucine at position 84 has been replaced with leucine; or proline and serine have been inserted in between the isoleucine at position 84 and the methionine at position 85.

In claim 1, the recitation of "a combination of (2) and (3)" should read "a combination of (1) and (2)".

Even though claim 9 depends on claim 1, the embodiment of the mutant prenyl diphosphate synthase according to claim 9 wherein proline and serine have been inserted in between the isoleucine at position 84 and the methionine at position 85 of the geranylgeranyl diphosphate synthase set forth in SEQ ID NO:1 (please see the last three lines of claim 9 reproduced above, or the last two lines of claim 9 printed in the '832 patent) is NOT covered by claim 1. Applicants note that in the amino acid sequence of geranylgeranyl diphosphate synthase set forth in SEQ ID NO:1, position 82 corresponds to D_1, position 83 corresponds to D_2 and position 86 corresponds to D_3 of the sequence, $D_1D_2X_1X_2(X_3X_4)D_3$, recited in claim 1. The embodiment of the mutant wherein "proline and serine have been inserted in

between the isoleucine at position 84 and the methionine at position 85" of the geranylgeranyl diphosphate synthase set forth in SEQ ID NO:1 is not covered by claim 1 of the '832 patent because the two amino acids are inserted between position 84 and position 85, and such an insertion is

NOT (1) at least one amino acid substitution, said at least one amino acid substitution located at least one amino acid position selected from (a) an amino acid between D_1 and the amino acid residue at the fifth position upstream of D_1 and (b) the amino acid residue located one amino acid position upstream of D_3; and

NOT (2) at least one additional amino acid inserted between D_3 and the first amino acid upstream of D_3, recited in claim 1.

Regarding limitation (2), at least one additional amino acid inserted between D_3 and the first amino acid upstream of D_3, as recited in claim 1, applicants note that the first amino acid upstream of D_3 is position 85 if the amino acid sequence of SEQ ID NO:1 is considered as the wild type prenyl diphosphate synthase. In contrast, in the embodiment of the mutant according to claim 9, the proline and serine are inserted between position 84 and position 85, not between position 85 and position 86 as required by limitation (2) of claim 1.

As a result, the '832 patent is partly inoperative or invalid at least by reason of the applicants claiming less than they had the right to claim in claim 1 of the patent.

Furthermore, independent claim 1 of the '832 patent is directed to a mutant prenyl diphosphate synthase which can have an amino acid substitution located one position upstream of D_3. However, certain advantageous embodiments of the invention that are disclosed in the '832 patent specification are not, through inadvertant error, specifically claimed in the '832 patent. For example, particular mutant prenyl diphostate synthases which have an amino acid substitution "located two positions upstream of D_3" are described in the specification but the claims of the '832 patent do not explicitly include this language. Accordingly, newly added claims 19-34 are presented in this reissue application to clarify the patent coverage to which Applicants are entitled.

The claims added in one of the amendments mentioned above and the support therefor are set forth as follows for the examiner's convenience.

Claim 19. A mutant prenyl diphosphate synthase having a modified amino acid sequence, wherein

said mutant diphosphate synthase comprises an aspartic acid-rich domain having the sequence, $D_1D_2X_1(X_2X_3)X_4D_3$, in region II of said mutant prenyl diphosphate synthase,

wherein each of D_1, D_2, and D_3 denote an aspartic acid residue; X_1, X_2, X_3, and X_4 are each independently any amino acid and X_2 and X_3 are each optionally independently present in the aspartic acid rich domain, and wherein

said mutant prenyl disphosphate synthase comprises (1) at least one amino acid substitution, said at least one amino acid substitution located at least one amino acid position selected from (a) an amino acid between D_1 and the amino acid residue at the fifth position upstream of D_1 and (b) the amino acid residue located one amino acid position downstream of D_2; (2) at least one additional amino acid inserted between the first amino acid downstream of D_2 and the first amino acid upstream of D_3; or (3) a combination of (1) and (2);

wherein said mutant prenyl diphosphate synthase synthesizes prenyl diphosphate which is shorter than prenyl diphosphate synthesized by a corresponding wild-type enzyme.

Applicants submit that they are entitled to subject matter relating to "(b) the amino acid residue located one amino acid position downstream of D_2" because this subject matter is distinct from that of Claim 1 and mutant enzymes containing mutations located one amino acid position downstream of D_2 are described in the specification.

As taught by the specification, a position located one amino acid position downstream of D_2 is position 84 in the wild type *Sulfolobus acidocaldarius* enzyme (*compare* the formula $D_1D_2X_1(X_2\ X_3)X_4\ D_3$ with the Feature identified by SEQ ID NO:

1). The specification teaches that the isoleucine present at position 84 (X_1) in the wild type *Sulfolobus acidocaldarius* enzyme can, according to the present invention, be substituted with another amino acid. *See* '832 Patent, Col. 6, lines 35-44; Examples 2 and 4. In "Mutant enzyme 5", the specification teaches that leucine can be substituted for the isoleucine at position 84. *See* '832 Patent, Col. 6, lines 59-64; *see also* SEQ ID NOS: 13 and 8 (showing that a codon for isoleucine (ATT and ATC) can be replaced with a codon for leucine (CTT)).

Furthermore, the specification teaches that amino acids can be inserted between amino acid position 84 (X_1) and amino acid position 85 (X_4). In "Mutant enzyme 5", the specification teaches insertion of a proline (X_2) and serine (X_3) residue in between the isoleucine at position 84 (X_1) and the methionine at position 85 (X_4). Accordingly, applicants submit that the subject matter of Claim 19 is fully supported by the specification and that no new subject matter is being presented in this reissue application.

Claims 20-34 depend from independent Claim 19 and relate to the subject matter of issued claims 2-16. Accordingly, applicants submit that the subject matter of these claims is fully also supported by the specification and that no new subject matter is being presented in this reissue application.

Claim 20. A mutant prenyl diphospate synthase according to claim 19 wherein said mutant has the enzymatic activities and thermostability of wild type prenyl diphosphate synthase.

Claim 21. A mutant enzyme according to claim 19 wherein the reaction product of the prenyl diphosphate synthase is farnesyl diphosphate.

Claim 22. A mutant enzyme according to claim 19 wherein the prenyl diphosphate synthase is a homodimer.

Claim 23. A mutant enzyme according to claim 19 wherein the prenyl diphosphate synthase is derived from archaea.

Claim 24. A mutant enzyme according to claim 19 wherein the prenyl diphosphate synthase is derived from *Sulfolobus acidocaldarius*.

Claim 25. A mutant enzyme according to claim 19 wherein the prenyl diphosphate synthase is a thermostable enzyme.

Claim 26. A mutant prenyl diphosphate synthase according to claim 19, wherein at least one amino acid selected from phenylalanine at position 77, threonine at position 78, valine at position 80, histidine at position 81, and isoleucine at position 84 has been substituted by another amino acid, or one or more amino acids have been inserted in between isoleucine at position 84 and methionine at position 85 in the geranylgeranyl diphosphate synthase as set forth in SEQ ID No:1.

Claim 27. A mutant prenyl diphosphate synthase according to claim 19 wherein at least one amino acid selected from phenylalanine at position 77, threonine at position 78, valine at position 80, histidine at position 81, and isoleucine at position 84 has been substituted by another amino acid, and/or two amino acids have been inserted between isoleucine at position 84 and methionine at position 85 in the geranylgeranyl diphosphate synthase as set forth in SEQ ID No: 1, wherein the phenyl alanine at position 77 has been replaced with tyrosine, the threonine at position 78 has been replaced with phenylalanine or serine, the valine at position 80 has been replaced with isoleucine, the histidine at position 81 has been replaced with leucine or alanine, or the isoleucine at position 84 has been replaced with leucine; or proline and serine have been inserted in between the isoleucine at position 84 and the methionine at position 85.

Claim 28. A mutant prenyl diphosphate synthase according to claim 19, wherein the mutant prenyl diphosphate synthase is derived from a native geranylgeranyl diphosphate synthase of an organism selected from the group consisting of *Arabidopsis thaliana, Lupinas albus, Capsicum annuum, Sulfolobus acidocaldarius, Rhodobactor sphaeroides, Rhodobacter capsulatus, Erwinia herbicola, Myxococcus thaliana and Neurospora crassa.*

Claim 29. A DNA encoding an enzyme according to claim 19.

Claim 30. An RNA transcribed from a DNA according to claim 29.

Claim 31. A recombinant vector comprising a DNA according to claim 29.

Claim 32. A host organism transformed with a recombinant vector according to claim 31.

Claim 33. A process for producing a mutant enzyme according to claim 19, said method comprising the steps of culturing a host transformed with an expression vector comprising a DNA coding for the mutant enzyme and harvesting the expression product from the culture.

Claim 34. A process for producing a prenyl diphosphate having not more than 15 carbons comprising the step of bringing an enzyme according to claim 19 into contact with a substrate selected from the group consisting of isopentenyl diphosphate, dimethylallyl diphosphate, and geranyl diphosphate.

8. Furthermore, inadvertant typographical errors are present in the '832 specification. These errors are corrected in the amendments provided below.

A. At col. 1, line 15, please delete the "s" from "unit[s]". The paragraph containing this text is reproduced below, with the correction identified.

> Of the substances having important functions in organisms, many are biosynthesized using isoprene (2-methyl-1,3-butadiene) as a constituent unit[s]. These compounds are also called isoprenoids, terpenoids, or terpenes, and are classified depending on the number of carbon atoms into hemiterpenes (C5), monoterpenes (C10), sesquiterpenes (C15), diterpenes (C20), sesterterpenes (C25), triterpenes (C30), tetraterpenes (C40), and the like. The actual biosynthesis starts with

the mevalonate pathway through which mevalonic acid-5-diphosphate is synthesized, followed by the synthesis of isopentenyl diphosphate (IPP) which is an active isoprene unit.

B. At col. 1, line 45, please delete the "plants" and at col. 1, line 46, after "in" please insert "plants." The paragraph containing this text is reproduced below, with the corrections identified.

There are Z type and E type condensation reactions. Geranyl diphosphate is a product of E type condensation and neryl diphosphate is of Z type condensation. Although, the all-E type is considered to be the active form in farnesyl diphosphate and geranylgeranyl diphosphate, the Z type condensation reaction leads to the synthesis of natural rubber, dolichols, bactoprenols (undecaprenols), and [plants] various polyprenols found in plants. They are believed to undergo the condensation reaction using the phosphate ester bond energy of the pyrophosphate and the carbon backbone present in the molecule and to produce pyrophosphate as the byproduct of the reaction.

C. At col. 2, line 4, please delete "[geraniols and that isomer nerol belonging]" and substitute "geraniol and its isomer, nerol, belonging " therefor. At col. 2, line 5, after "monoterpens" please insert "that." The paragraph containing this text is reproduced below, with the corrections identified.

Furthermore, via the biosynthesis of these active-form isoprenoids, a vast number of

kinds of compounds that are vital to life have been synthesized. Just to mention a few, there are cytokinins that are plant hormones and isopentenyl adenosine-modified tRNA that use hemiterpenes as their precursor of synthesis, [geraniols and that isomer nerol belonging] geraniol and its isomer, nerol, belonging to monoterpens that are the main components of rose oil perfume and a camphor tree extract, camphor, which is an insecticide. Sesquihormones include juvenile hormones of insects, diterpenes include a plant hormone gibberellin, trail pheromones of insects, and retinols and retinals that function as the visual pigment precursors, binding components of the purple membrane proteins of highly halophilic archaea, and vitamin A.

D. At col. 3, line 45, please delete "[(DDXX(XX)D)]" and substitute "$(D_1D_2X_1(X_2X_3)X_4D_3)$" therefore. At col. 3, line 56, please delete the "[have not been]" and substitute "are" therefor. At col. 3, line 57, please delete the "[that induce mutation]" and substitute "that include mutations" therefor. At col. 3, lines 58-59, please delete "[to be in the short chain-length side]" and substitute with having a shorter chain length. The paragraph containing this text is reproduced below, with the corrections identified.

It has been found out that of the two aspartic acid-rich domains that have been proposed based on the amino acid sequence of the prenyl diphosphate synthase, the amino acid residue located at the fifth position in the N-terminal direction

from the conserved sequence I [(DDXX(XX)D)] ($D_1D_2X_1(X_2X_3)X_4D_3$) (wherein X denotes any amino acid, and the two X's in the parentheses may not be present) of the aspartic acid-rich domain in the amino-terminal side is responsible for controlling the chain length of the reaction product. Hence, a method has been invented that controls the reaction product for the purpose of lengthening the chain length of the reaction product [Japanese patent application No. 8-191635 filed on Jul. 3, 1996 under the title of "A Mutant Prenyl Diphosphate Synthase"]. The enzyme produced using the method enables production of reaction products that have several chain lengths. However, methods [have not been] are not known [that induce mutation] that include mutation of geranylgeranyl diphosphate synthase to control the reaction products [to be in the short chain-length side] having a shorter chain length in order to produce farnesyl diphosphate.

E. At col. 4, line 2, please delete the "[owned by the]" and substitute "exhibited by" therefor. The paragraph containing this text is reproduced below, with the corrections identified.

It is an object of the invention to establish a process for producing farnesyl diphosphate synthases by modifying amino acid sequences of prenyl diphosphate enzymes. A new enzyme that is more stable or that has a high specific activity more adaptable to industrial application would make it possible to

obtain immediately a mutant prenyl diphosphate synthase or the gene thereof that produces farnesyl diphosphate and that retains the property [owned by the] exhibited by the prenyl diphosphate synthase prior to mutation.

F. At col. 4, line 11, please delete "[(DDXX(XX)D)]" and substitute "$(D_1D_2X_1(X_2X_3)X_4D_3)$" therefore. The paragraph containing this text is reproduced below, with the corrections identified.

From the information on the nucleotide sequence of the gene of the geranylgeranyl diphosphate synthase of the mutant Sulfolobus acidocaldarius (S. acidocaldarius), it was clarified that out of the two Aspartic acid-rich domains that have been proposed based on the analysis of the amino acid sequence of prenyl diphosphate synthases, the amino acid residues within the aspartic acid-rich domain conserved sequence I [(DDXX(XX)D)] $(D_1D_2X_1(X_2X_3)X_4D_3)$ at the amino terminal side or the five amino acid residues to the N-terminal side from the amino terminal of said conserved sequence I are involved in the control of chain length of the reaction products.

G. At col. 4, line 23, please delete "[(DDXX(XX)D)]" and substitute "$(D_1D_2X_1(X_2X_3)X_4D_3)$" therefor. At col. 4, line 26, please delete "[at the position in the N-terminal direction from D of the C-terminal of said aspartic acid-rich domain]" and substitute "one amino acid position downstream of D2" therefor. The paragraph containing this text is reproduced below, with the corrections identified.

at least one amino acid residue selected from (a) the amino acid residues in between the amino acid residue located at the fifth position in the N-terminal direction from D of the N-terminal and the amino acid residue located at the first position in the N-terminal direction from D of said N-terminal of the aspartic acid-

rich domain [DDXX(XX)D] ($\underline{D_1D_2X_1(X_2X_3)X_4D_3}$) (wherein X sequence denotes any amino acid, and the two X's in the parentheses may not be present) present in region II, and (b) the amino acid residue located one amino acid position downstream of D2 [at the position in the N-terminal direction from D of the C-terminal of said aspartic acid-rich domain] has been substituted by another amino acid, and/or

H. At col. 4, line 32, please delete "[amino acid residues located at the first position in the N-terminal direction from D of the C-terminal and D of said C-terminal]" and substitute "first amino acid downstream of D_2 and the first amino acid upstream of D_3." therefor. The paragraph containing this text is reproduced below, with the corrections identified.

additional amino acid(s) have been inserted in between the first amino acid downstream of D_2 and the first amino acid upstream of D_3 [amino acid residues located at the first position in the N-terminal direction from D of the C-terminal and D of said C-terminal] of said aspartic acid-rich domain.

I. At col. 5, line 36, please delete "[(DXX(XX)D)]" and substitute "($\underline{D_1D_2X_1(X_2X_3)X_4D_3}$)" therefore. The paragraph containing this text is reproduced below, with the corrections identified.

It has been proposed that there are five conserved regions in the amino acid sequence of a prenyl diphosphate synthase (one subunit in the case of a heterodimer) [A. Chem et al., Protein Science Vol. 3, pp. 600-607, 1994]. It is also known that of the five conserved regions, there is an aspartic acid-rich domain conserved sequence [[DDXX(XX)D]] $\underline{D_1D_2X_1(X_2X_3)X_4D_3}$ (wherein X denotes any amino acid, and the two X's in the parentheses may not be present) in

region II. Although there is also an aspartic acid-rich domain indicated as "DDXXD" in region V, the aspartic acid-rich domain used to specify the modified region of the amino acid sequence of the present invention is the one present in region II, and this domain is termed as the aspartic acid-rich domain I as compared to the aspartic acid-rich domain II present in region V.

J. At col. 6, line 6, please delete "[(DDXX(XX)D)]" and substitute "($\underline{D_1D_2X_1(X_2X_3)X_4D_3}$)" therefore. At col. 6, line 8, please delete "[at the position in the N-terminal direction from D of the C-terminal of said aspartic acid-rich domain]" and substitute "one amino acid position downstream of D2" therefor. The paragraph containing this text is reproduced below, with the corrections identified.

In accordance with the present invention, in the amino acid sequence of a prenyl diphosphate synthase, at least one amino acid residue selected from (a) the amino acid residues in between the amino acid residue located at the fifth position in the N-terminal direction from D of the N-terminal and the amino acid residue located at the first position in the N-terminal direction from D of said N-terminal of the aspartic acid-rich domain [DDXX(XX)D] $\underline{D_1D_2X_1(X_2X_3)X_4D_3}$ (wherein X sequence denotes any amino acid, and the two X's in the parentheses may not be present) present in region II, and (b) the amino acid residue located one amino acid position downstream of D2 [at the position in the N-terminal direction from D of the C-terminal of said aspartic acid-rich domain] has been substituted by another amino acid, and/or

K. At col. 6, line 16, lease delete "[amino acid residues located at the first position in the N-terminal direction from D of the C-terminal and D of said C-

terminal]" and substitute "first amino acid downstream of D_2 and the first amino acid upstream of D_3-" therefor. The paragraph containing this text is reproduced below, with the corrections identified.

> an additional amino acid(s) have been inserted in between the first amino acid downstream of D_2 and the first amino acid upstream of D_3 [amino acid residues located at the first position in the N-terminal direction from D of the C-terminal and D of said C-terminal] of said aspartic acid-rich domain.

L. At col. 7, line 67, please delete "[biding]" and substitute "binding" therefor. The paragraph containing this text is reproduced below, with the corrections identified.

> It is known that the distance between the sequence of the ribosome [biding] binding site (GGAGG and similar sequences thereof) and the initiation codon ATG is important as the sequence regulating the ability of synthesizing protein from mRNA. It is also well known that a terminator (for example, a vector containing rrn PT_1 T_2 is commercially available from Pharmacia) that directs transcription termination at the 3'-end affects the efficiency of protein synthesis by a recombinant.

M. At col. 9, line 14, please delete "[a]" and substitute "an" therefor. At col. 9, line 16, please delete "[prrenyl]" and substitute "prenyl " therefor. The paragraph containing this text is reproduced below, with the correction identified.

> By using the method of producing the mutant prenyl diphosphate synthase obtained by the present invention, the

mutant prenyl diphosphate synthase derived from [a] an archaea may be created that is more stable and thus easier to handle and that produces [prrenyl] prenyl diphosphate. Furthermore, there is also expected a creation of the farnesyl diphosphate-producing mutant prenyl diphosphate synthase that has the property of the prenyl diphosphate synthase prior to mutation (for example, salt stability or stability in a wide range of pH) added thereto.

N. At col. 10, line 4, please delete "[Geranylaeranyl]" and substitute "Geranylgeranyl" therefor. The paragraph containing this text is reproduced below, with the corrections identified.

Construction of a Plasmid Containing the Gene for [Geranylaeranyl] Geranylgeranyl Diphosphate Synthase

O. At col. 10, line 57, please delete "[TATT-31]" and substitute "TATT-3' " therefor. The paragraph containing this text is reproduced below, with the corrections identified.

Introduction of the mutation (F77Y, T78S, V80I, I84L, 84PS85) was effected using two nucleotides. First, mutation was introduced as mentioned in Example 3 using the oligonucleotide

5'-GTTCTTCATACTTATTCGCTTATTCATGATAG [TATT-31] TATT-3' (SEQ ID No: 7) and a transformant was prepared in accordance with Example 4, and furthermore mutation

was introduced into the plasmid thus obtained using the oligonucleotide

5'-ATTCATGATGATCTTCCATCGATGGAT CAAGAT-3' (SEQ ID No: 8).

P. At col. 11, line 24, please delete "[H2O]" and substitute "$\underline{H_2O}$ " therefor. The paragraph containing this text is reproduced below, with the corrections identified.

[H2O] $\underline{H_2O}$ 5 µl

Q. At col. 11, line 56, please delete "[H2O]" and substitute "$\underline{H_2O}$ " therefor. The paragraph containing this text is reproduced below, with the corrections identified.

[H2O] $\underline{H_2O}$ make to a final volume of 10 µl

R. At col. 12, line 35, please delete "[ATATCATG-31]" and substitute "ATATCATG-3' " therefor. The paragraph containing this text is reproduced below, with the corrections identified.

F77Y, T78F, H81L:
5'-TATTTCCTTGTGCTTGATG
[ATATCATG-31] ATATCATG-3' (SEQ ID No: 11)

9. All errors corrected in this reissue application arose without any deceptive intention on the part of the applicants.

10. We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001, and that such willful false

statements may jeopardize the validity of the application or any patent issuing thereon, or any patent to which this declaration is directed.

Full name of the first inventor (given name, family name)

Hiroyuki Nakane

Inventor's Signature: Hiroyuki Nakane

Date: May 19, 2009

Residence: Toyota-shi, Aichi, Japan

Citizenship: Japan

Post Office Address: 70-2, Baba, Iwakura-cho, Toyota-shi, Aichi, Japan

Full name of the second inventor (given name, family name)

Chikara Ohto

Inventor's Signature: Chikara Ohto

Date: May 19, 2009

Residence: Toyota-shi, Aichi, Japan

Citizenship: Japan

Post Office Address: Noburuaifumurama 501, 3-21-1, Kanaya-cho, Toyota-shi, Aichi, Japan

Full name of the third inventor (given name, family name)

Shinichi Ohnuma

Inventor's Signature: Shinichi Ohnuma

Date: June 30 2009

Residence: Sendai-shi, Miyagi, Japan

Citizenship: Japan

Post Office Address: Regidensuhirose 102, 48-1, Kawauchi Kawamae-cho, Aoba-ku, Sendai-shi, Miyagi, Japan

Full name of the fourth inventor (given name, family name)

Kazutake Hirooka

Inventor's Signature: Kazutake Hirooka

Date: June 25, 2009

Residence: Sendai-shi, Miyagi, Japan

Citizenship: Japan

Post Office Address: 1-30-310, Sakuragi-cho, Taihaku-ku, Sendai-shi, Miyagi, Japan

Full name of the fifth inventor (given name, family name)

Tokuzo Nishino

Inventor's Signature: Nishino

Date: July 13, 2009

Residence: Sendai-shi, Miyagi, Japan

Citizenship: Japan

Post Office Address: 2-15-3, Minamiyoshinari, Aoba-ku, Sendai-shi, Miyagi, Japan